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Original Article

The interaction of serum leptin with brain serotonin in an animal model of diet-restriction-induced anorexia nervosa

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ABSTRACT

Leptin is a peptide hormone regulating body weight, appetite control and energy homeostasis. Plasma leptin levels rapidly decrease after body weight loss in human and in rodent. Studies suggested close interactions between leptin and the serotonergic system. The serotonergic system is involved in the regulation of appetite and mood and is a major biological system of interest in research on Anorexia Nervosa (AN). The present study is designed to investigate the interaction of serum leptin with brain serotonin in an animal model of Diet Restriction (DR) induced AN. Serum leptin and corticosterone were measured by ELISA. Tryptophan, 5-HT and 5-HIAA concentrations were determined by HPLC-EC in the hypothalamus. Animals of DR group given access to food 2 h daily for 5 weeks exhibited 20.4% decreased in body weight compared to Freely Fed (FF) group. Animal exposed in open field, activity box and light dark transition test exhibited hyperactivity and anxiety/depression like behaviors. The levels of serum leptin and TRP were significantly decreased in diet restricted group. The 5-HT metabolism was significantly decreased in the hypothalamus in DR group. The interaction of serum leptin with brain serotonin in DR-induced AN is discussed.

Keywords: leptin, tryptophan, corticosterone, 5-HT, Diet Restriction, anorexia nervosa

INTRODUCTION

Serotonin, 5-hydroxytrypamine (5-HT), known to play a role in feeding behavior as an anorectic molecule [1]. Studies showed the involvement of 5-HT in feeding and satiety and decreased food consumption. Thus, serotonergic system has been a viable target in weight control. Neurochemical studies on the effects of both starvation and feeding shows increased levels of 5-HT metabolism in the brain. [2]. Carbohydrate ingestion enhances the, availability of tryptophan to the brain for the synthesis and release of hypothalamic 5-HT. The increase in brain 5-HT metabolism is thought to generate signal for the termination of meal .TRP the precursor of 5 HT is an essential amino acid, its source is only dietary. Considerable amounts of evidence from animal and healthy human studies show that a restricted diet significantly lowers plasma TRP[3], resulting in a decreased plasma ratio of TRP to large neutral amino acids, and, in turn, a reduction in the availability of TRP to the brain. Under weight anorectics patients exhibit low basal levels of 5-HIAA, major metabolite of 5-HT in the CSF, which returned to normal after weight recovery [4, 5]. It is possible that DR induces decreases of 5-HT provoke compensatory upregulation of post synaptic hypophagic 5-HT receptors to precipitate AN.

Alterations in certain physiological parameters trigger food restriction and hyperactivity in AN [6-7]. Leptin is one of the peripheral signaling molecules regulates body weight, appetite control and energy homeostasis [8-9]. Studies suggested that plasma leptin levels rapidly decreases after body weight loss in human and in rodents Studies suggested close interactions between leptin and the serotonergic system. The present study is therefore, designed to investigate the role of 5-HT in the brain

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particularly in the hypothalamus. The hypothalamus is known to have a key role in the regulation of food intake and is believed to be the site of brain transducing satiety signals of serotonin. The interaction of serum leptin with serotonin in an animal model of Diet Restriction (DR) induced anorexia nervosa is also discussed.

MATERIALS AND METHODS

Animals and Treatment: Locally bred female Sprague-Dawley rats weighing 191.5g ± 12 were housed individually under 12 h light dark cycle and controlled room temperature (22±2ºC) with free access to cubes of standard rodent diet and water for at least three days before experimentation. Animals were cared according to a protocol approved locally which is consistent with the NIH guidelines for the care and use of laboratory animals.

Experimental protocol: One week after arrival twelve (12) female rats were randomly divided into Freely Feeding (FF) and Diet Restriction (DR) group of 6 each. Food was available for 24 h to FF group while animals of DR group were given access to food daily for 2 h. Food intake and body weights of the two groups were monitored weekly. Behavioral activities were also monitored weekly. The animals were decapitated after week 5 between 10:00 and 11:00 h. Blood was allowed to clot at 4°C and serum was separated and stored at -80°C for biochemical estimations of leptin and corticosterone by ELISA. Brains removed rapidly were dipped in ice cold saline. Hypothalami dissected out as described previously [10] were stored at -80 ºC until analysis by HPLC–EC.

Neurochemical Analysis: TRP, 5-HT and 5-HIAA were determined by HPLC – EC as described before [10]. A5II Shim-Pack ODS separation columns of 4.0 mm internal diameter and 15 cm length were used. Separation was achieved by a mobile phase containing methanol (14%), Octyl sodium sulphate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer of pH 2.9 at an operating pressure of 2000 -3000 psi on Schimadzu HPLC-EC 6A detector at an operating potential of 1.0 volts for TRP.

Statistical analysis: Results are represented as means ±S.D. Behavioral data were analyzed by one-way ANOVA (repeated measure design). Neurochemical and Biochemical data were analyzed by *t*-test. Post hoc comparisons done by Tukey's test. p values < 0.05 were taken significant. SPSS version 16 was used to analyze data.

RESULTS

Food intakes of rats fed on DR schedule of 2 h/day for five week and FF rats are shown in Figure 1. One-way ANOVA (repeated measure design) showed significant effect of DR (df. 4, 10, F = 664.274; $p < 0.01$); interaction, (df. 1, 10, F= 711.2; $p<0.01$); diet (df. 1, 10, F= 49.158; $p<0.01$) , whereas, effects of repeated monitoring on week × diet (df. 4, 10, F= 0.415; $p>0.01$) was insignificant. Post hoc test showed that food intakes were significantly ($p < 0.01$) decreased after week 1 to week 5 in DR than FF group.

Body weight changes in FF and DR groups during five week treatment are shown in Figure 2. One-way ANOVA (repeated measure design) showed significant effect of DR (d.f. 4, 10F = 25.89; $p < 0.01$). Interaction, (df. 1, 10, F= 217.6; $p<0.01$); diet (df. 1, 10, F= 4.4; $p>0.01$) was insignificant and effects of repeated monitoring on week × diet (df. 4, 10, F= 11.142; $p>0.01$) was also significant. Post hoc test showed that body weights were significantly ($p < 0.01$) decreased after week 1 to week 5 in DR than FF group.

DR induced hyperactivity in FF and DR group are shown as in Figure 3. One way ANOVA (repeated measures design) showed significant effect of DR (df 4, 10, F= 9.6; $p < 0.05$) , interaction, (df. 1, 10, F= 926.644; $p<0.01$);diet (df. 1, 10, F= 268.817; $p<0.01$) , whereas, effects of repeated monitoring on week × diet (df. 4, 10, F= 9.64; $p<0.01$) was also insignificant. Post hoc test showed that activity scores (number of cage crossings) were significantly increased. After week 4 ($p < 0.05$) and week 5 ($p < 0.05$) than FF group. The increase in hyperactivity in DR group from week 1 to week 3 was insignificant.

Effect of DR in an open field activity in FF and DR group are shown as in Figure 4. One way ANOVA (repeated measures design) showed significant effect of DR (df 4, 10,F= 17.8 ; $p < 0.05$) ,whereas, interaction, (df. 1, 10, F= 720.1; $p<0.01$);diet (df. 1, 10, F= 160.392; $p<0.01$) ,effects of repeated monitoring on week × diet (df. 4, 10, F= 17.182; $p<0.01$) were significant. Post hoc test showed that number of square crossed was significantly increased after week 4 ($p < 0.05$) and week 5 ($p < 0.05$) than FF group. The increase in number of square crossed in DR group from week 1 to week 3 was insignificant.

Effect of DR on light dark activity in FF and DR group are shown as in Figure 5. One way ANOVA (repeated measures design) showed significant effect of DR (df 4, 10,F=2.16 ; $p < 0.05$) , interaction (df. 1, 10, F= 521.145; $p<0.01$);diet (df.

1, 10, F= 7.446; p<0.05) were significant , whereas, effects of repeated monitoring on week × diet (df. 4, 10, F= 1.325; p>0.01) was insignificant. Post hoc test showed that time spent in light box was decreased after week 4 ($p < 0.05$) and week 5 ($p < 0.05$) than FF group. The decrease in DR group from week 1 to week 3 was insignificant.

Effects of DR on the levels of leptin and corticosterone in the serum of FF and DR rats are shown Figure 6. Independent sample *t*-test showed a significant (df=1, 10, t= 14.9, $p < 0.01$) decreased effect of DR on serum leptin levels Serum corticosterone levels was significantly (df=1,10, t=8.17, $p < 0.01$) increased in DR group when compared to FF group.

Effects of DR on the levels of TRP in Serum and TRP, 5-HT and5-HIAA in the hypothalamus of FF and DR rats are shown in Figures 7. Independent sample *t*-test showed significant (df=1, 10, t=2.35, $p < 0.05$) decreased effect of DR on serum TRP levels. Effects of DR on decreased TRP levels in the hypothalamus (df=1, 10, t= 18.6, $p < 0.01$) and for 5-HT(df=1,10, t= 3.5, $p < 0.05$) were significant. Effects of DR on 5-HIAA levels was insignificant (df=1, 10, t= 1.15, $p > 0.05$) in the hypothalamus.

DISCUSSION

In the present study rats fed on DR schedule of 2 h/day for five weeks exhibited decreased in body weight (20.4%;) (Fig 2), food intake, less time spent in light dark transition test and increased motor activity in activity box and open field. Similar (15-25%) reductions in body weight and behavioral deficits have also been reported in AN [10-11].

Studies in human and animal raise the possibility that alterations of 5-HT1A receptor function may play a role in anxiety [12-15], mood and impulse control [16-17] and feeding behavior [18-19]. 5-HT1A receptors are present on the soma and dendrites of the serotonergic neurons and also on post synaptic sites. PET imaging techniques has been shown that 5-HT1A receptor activity is increased in AN patients. In the present study the anxiety/depression like behavior in DR group may be due to an increased activity of 5-HT1A receptors [20]. The present study shows decreased 5-HT metabolism in the hypothalamus. (Fig.7). The levels of TRP also significantly decreased in the serum and in the hypothalamus. TRP the precursor of 5 HT is an essential amino acid, its source is only dietary. Considerable amounts of evidence from animal and healthy human studies show that a restricted diet significantly lowers plasma TRP, resulting in a decreased plasma ratio of TRP to neutral amino acids, and, in turn, a reduction in the availability of TRP to the brain. TRP-hydroxylase the rate limiting enzyme of 5-HT biosynthesis is only half saturated with its substrate. A change in the availability of substrate is one of the important factors that regulate 5-HT synthesis. In the present study it was observed that the decreases of serum and brain TRP might be due to smaller availability of TRP in DR group which could lead to decrease in 5-HT turnover in the brain in DR-induced AN in rats. Leptin receptors were cloned and identified in the choroid plexus and hypothalamus, a region known to be involved in the regulation of appetite food and body weight. Several lines of studies reported that decreased plasma leptin levels, as a result of the food restriction, may represent the initial trigger for the increased activity levels in AN patients and in food-restricted rats [21]. Our data also shows decreased in leptin levels in DR rats as compared to FF rats.

Progressive fasting results in a rapid decline in leptin concentration and increasing cortisol levels in AN patients. Cortisol increases the activity of 2, 3 TRP deoxygenase which is the main metabolic enzyme of TRP causes the degradation along the kynurenine pathway and thereby regulates systemic tryptophan levels. A significant decreases in plasma tryptophan and ratio of tryptophan to competing amino acid in circulation also occurred in healthy volunteers kept on 3 weeks of dieting.[11,21],24 h starvation as well as food restriction for one week both increased hepatic tryptophan pyrrolase activity in rats[11,22-23].

The present study shows a significant decreased in serum leptin and an increased in corticosterone levels in DR group as compared to the FF group in rats. The greater decreases of serum tryptophan in DR group in the present study might be due to an increased activity of 2, 3 TRP deoxygenase in DR group of rats than FF group. The dopamine system has traditionally been considered crucial to the control of motor activity whereas serotonin is known to inhibit or enhance dopamine neurotransmission, acting, respectively, through 5-HT-2C/5-HT-2A and 5-HT-1A receptors [24-25]. The hypothesis that serotonin may be involved in the elicitation of hyperactivity in AN [11] was largely based on studies showing low basal levels of 5-HIAA in the CSF of AN patients that returned to normal after weight recovery [4-5], whereas DR for only 1 week also decreased the 5-HT content and synthesis in the rat brain [10]. It was shown that DR-induced decreases in 5-HT were at least in part explainable in terms of exaggerated feedback control over 5-HT synthesis and release through somatodendritic 5-HT-1A receptors [11], which

resulted in a decrease in the availability of 5-HT in regions receiving serotonergic input from the raphe nuclei. A decrease in the availability of 5-HT in the caudate nucleus, releasing dopamine neurotransmission from the inhibitory influence of serotonin, may elicit hyperactivity. In the present study significant increase in the activity in the activity box as well as in open field were observed in DR group when compared to the FF group in rats. The present study shows that 5-HT metabolism was decreased in the hypothalamus in DR than FF rats. Therefore, an increased in hyperactivity in DR rats than FF rats could be due to the stimulation of somatodendritic 5-HT1A receptor resulting in a decreased serotonin turnover in DR-induced AN in rats.

Conclusion: The present study therefore concluded that a significant increased in DR-induced hyperactivity in DR rats as compared to FF rats. could be due to decreased concentration of 5-HT in the brain. This results in an increased in the inhibitory influence of 5-HT on the activity of dopaminergic neurons possibly due to increase supersensitivity of 5-HTIA receptors. The DR induced decreases of 5-HTmetbolism may increase vulnerability of depression in AN in rats which may further aggravates by hypoleptinemia

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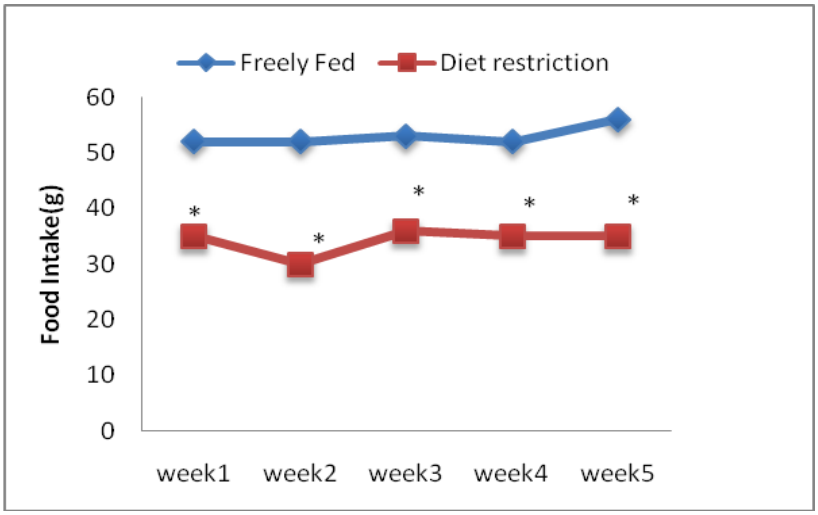

Figure 1. Food intake of FF and DR group of rats. Values are means ± S.D (n=6). Significant differences by Tukey's test. * p<0.01 from respective FF controls following one way ANOVA (repeated measure design).

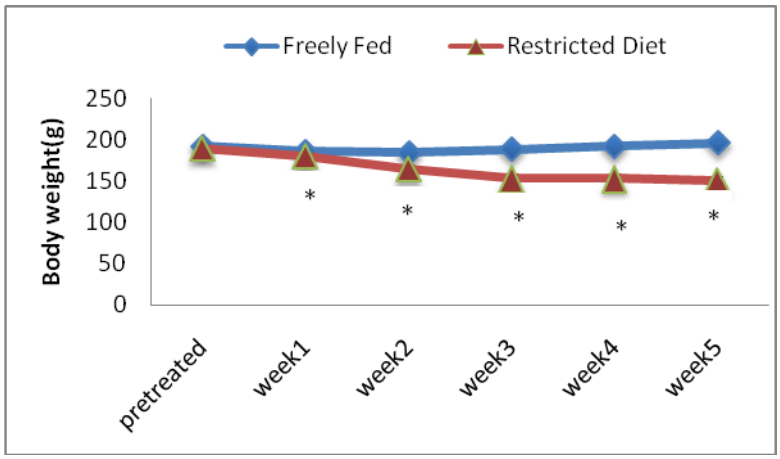

Figure 2. Body weight of FF and DR group of rats. Values are means ± S.D (n=6). Significant differences by Tukey's test. * p<0.01 from respective FF controls following one way ANOVA (repeated measure design).

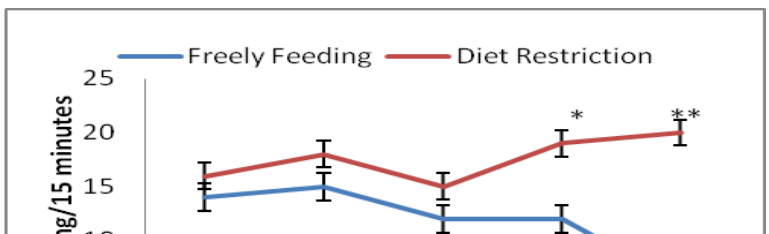

Figure 3. Activity in Activity box of FF and DR group of rats. Values are means ± S.D (n=6). Significant differences by Tukey's test. *p<0.05, ** p<0.01 from respective FF controls following one way ANOVA (repeated measure design).

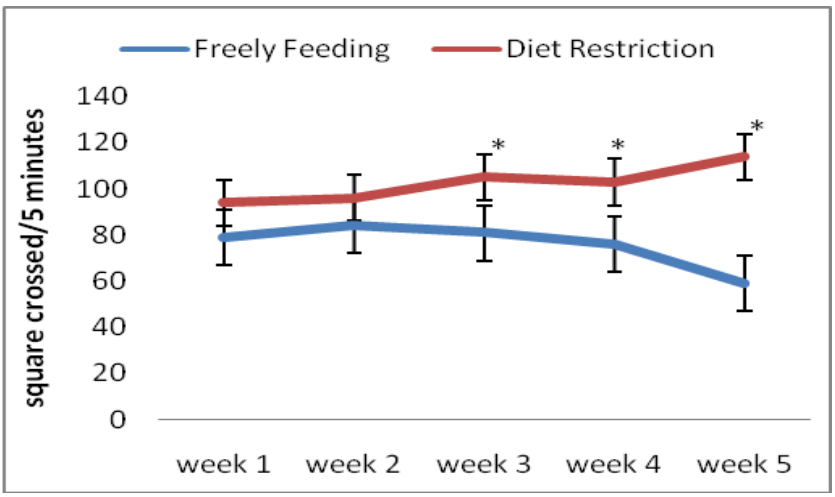

Figure 4. Activity in open field of FF and DR group of rats. Values are means ± S.D (n=6). Significant differences by Tukey's test. * p<0.01 from respective FF controls following one way ANOVA (repeated measure design).

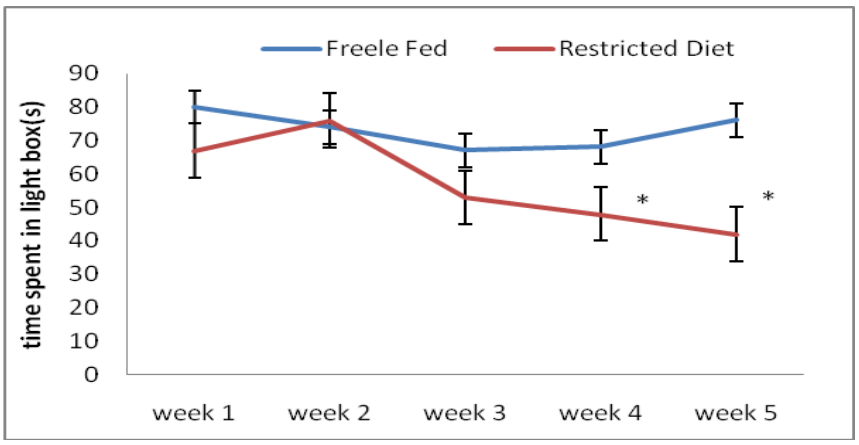

Figure 5. Activity in light dark box of FF and DR group of rats. Values are means ± S.D (n=6). Significant differences by Tukey's test. * p<0.05 from respective FF controls following one way ANOVA (repeated measure design).

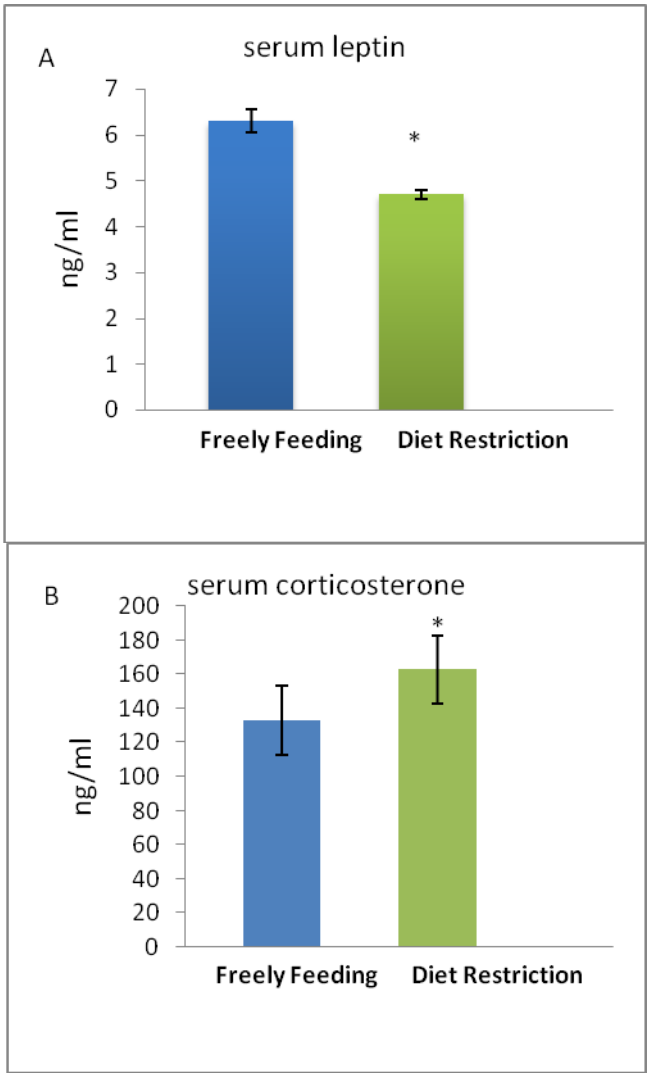

Figure 6. The levels of serum leptin (A) and corticosterone (B). Values are means ± S.D (n=6). Significant differences by t-test. * $p<0.01$ from respective FF group

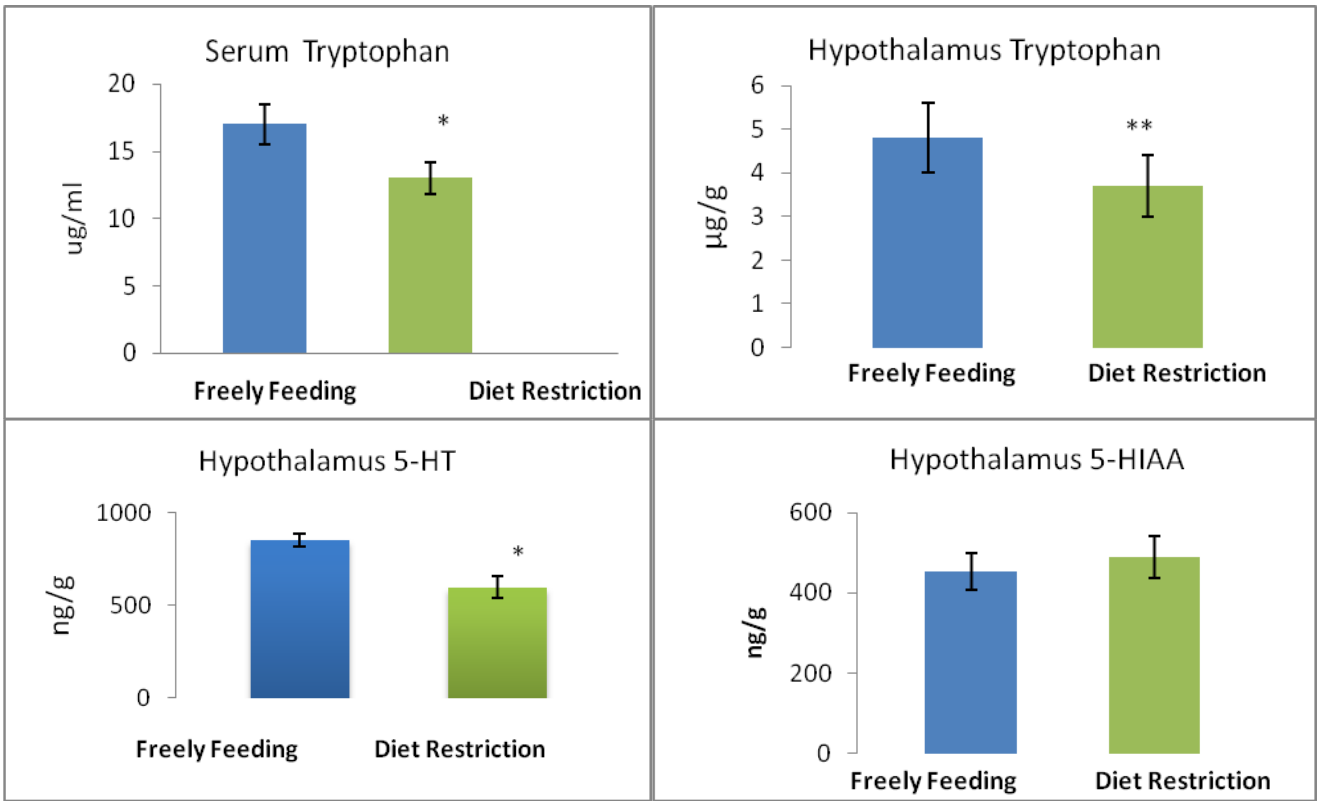

Figure 7. The levels of tryptophan, 5-HT and 5-HIAA. Values are means ± S.D (n=6). Significant differences by *t*-test. *p<0.05,** p<0.01 from respective FF group

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